

ANTICANCER DRUG DEVELOPMENT GUIDE

*PRECLINICAL SCREENING, CLINICAL TRIALS,
AND APPROVAL*

Edited by

BEVERLY A. TEICHER

Dana-Farber Cancer Institute, Boston, MA



HUMANA PRESS
TOTOWA, NEW JERSEY

*For the beautiful ones
Emily and Joseph*

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Totowa, New Jersey 07512

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ANSI Z39.48-1984 (American Standards Institute) Permanence of Paper for Printed Library Materials.

Cover illustration: From Fig. 1 in Chapter 14, "Discovery of TNP-470 and Other Angiogenesis Inhibitors," by Donald E. Ingber, in *Cancer Therapeutics: Experimental and Clinical Agents*, Edited by Beverly A. Teicher, Humana Press, 1997.

Cover design by Patricia F. Cleary.

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Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging-in-Publication Data

Anticancer drug development guide: preclinical screening, clinical trials, and approval / edited by
Beverly A. Teicher.

p. cm. -- (Cancer drug discovery and development)

Includes index.

ISBN 0-89603-461-5 (alk. paper)

I. Antineoplastic agents--Testing. I. Teicher, Beverly A., 1952--

II. Series.

[DNLN: 1. Antineoplastic Agents--standards. 2. Drug Approval. 3. Drug Screening. QV 269 A62953 1997]

RC271.C5A6722 1997

616.99'4061--dc21

DNLN/DLC

for Library of Congress

96-38113

CIP

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Human Tumor Xenograft Models in NCI Drug Development

*Jacqueline Plowman, PhD,
Donald J. Dykes, BS,
Melinda Hollingshead, PhD,
Linda Simpson-Herren, BS,
and Michael C. Alley, PhD*

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SUMMARY

1. INTRODUCTION

The preclinical discovery and development of anticancer drugs by the NCI consist of a series of test procedures, data review, and decision steps that have been summarized recently (1). Test procedures are designed to provide comparative quantitative data, which in turn, permit selection of the best candidate agents from a given chemical or biological class. Periodic, comprehensive reviews by various NCI committees serve not only to identify and expedite the development of active lead compounds that may provide more efficacious treatments for human malignancy, but also to eliminate agents that are inactive and/or highly toxic from further consideration.

Various components in NCI's drug discovery and development process have evolved in response to a combination of factors—scientific, clinical, technological, and fiscal. A series of review articles have charted the evolution of the drug screening program and have described specific elements of the process, e.g., acquisition, screening, analog development and testing, pharmacology, and toxicology (2-12). The present chapter provides: a brief history of the *in vivo* screens used by NCI; a description of the human tumor xenograft systems, which are currently employed in preclinical drug development; a discussion of how these xenograft models are employed for both initial efficacy testing as well as detailed drug evaluations; and a description of a new model that may facilitate preclinical drug development.

From: *Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval*
Edited by: B. Teicher Humana Press Inc., Totowa, NJ

2. HISTORICAL DEVELOPMENT OF NCI SCREENS

Analyses of various screening methods available prior to 1955 indicated that (1) nontumor systems were incapable of replacing tumor systems as screens, and (2) no single tumor system was capable of detecting all active antitumor compounds (13). Since that time, the preclinical discovery and development of potentially useful anticancer agents by the NCI have utilized a variety of animal and human tumor models not only for initial screening, but also for subsequent studies designed to optimize antitumor activity of a lead compound or class of compounds. Although the various preclinical data review steps and criteria have remained essentially the same throughout the years, the modes and rationale of in vivo testing employed by NCI have evolved significantly.

2.1. Murine Tumor Screens, 1955–1975

In 1955, NCI initiated a large-scale in vivo anticancer drug screening program utilizing three murine tumor models: sarcoma 180, L1210 leukemia, and carcinoma 755. By 1960, in vivo drug screening was performed in L1210 and in two additional rodent models selected from a battery of 21 possible models. In 1965, screening was limited to the use of two rodent systems, L1210 and Walker 256 carcinosarcoma. In 1968, synthetic agents were screened in L1210 alone, whereas natural product testing was conducted in both L1210 and P388 leukemias. A special testing step was added to the screen in 1972 to evaluate active compounds against B16 melanoma and Lewis lung carcinoma. It is noteworthy that this first 20 years of in vivo screening relied heavily on testing conducted in the L1210 model.

2.2. Prescreen and Tumor Panel, 1976–1986

In late 1975, NCI initiated a new approach to drug discovery that involved prescreening of compounds in the ip-implanted murine P388 leukemia model, followed by evaluation of selected compounds in a panel of transplantable tumors (14). The tumors in the panel were chosen as representative of the major histologic types of cancer in the US and, for the first time in NCI history, included human solid tumors. The latter was made possible through the development of immunodeficient athymic (nu/nu) mice and transplantable human tumor xenografts in the early 1970s (15,16). Beginning in 1976, the tumor panel consisted of paired murine and human tumors of breast (CD8F₁ and MX-1), colon (colon 38 and CX-1 [the same as HT29]) and lung (Lewis and LX-1), together with the B16 melanoma and L1210 leukemia used in previous screens.

The majority of the early NCI testing conducted with the human tumors used small fragments growing under the renal capsule of athymic mice. The subrenal capsule (src) technique and assay were developed by Bogden and associates (17). Although labor-intensive, the src assay provided a rapid means of evaluating new agents against human tumor xenografts at a time when the testing of large numbers of compounds against sc xenografts seemed untenable. As experience was gained with the husbandry of athymic mice, longer-duration sc assays became manageable.

A detailed evaluation of the sensitivities of individual tumor systems employed from 1976–1982 revealed a wide range in sensitivity profiles as well as “yield” of active compounds (14). The data clearly indicated that rodent models may not be capable of detecting all compounds with potential activity against human malignan-

cies, and indicated that the best strategy for testing is to employ a combination of tumor systems to minimize loss of potentially useful compounds. These findings prompted the NCI in 1982 to develop a strategy for testing compounds that involved a sequential process of "progressive selection": NCI continued to use the P388 leukemia as a prescreen, but subsequent evaluation of selected agents was conducted in a modified tumor panel composed of "high-yield" models from the original panel (i.e., src-implanted MX-1 mammary carcinoma, and ip-implanted B16 melanoma and L1210 leukemia) and a new model, the ip-implanted M5076 sarcoma. Thereafter, evaluation of selected compounds would be "compound-oriented" and use protocols and models, selected on the basis of prior testing results and known properties of each compound, that would present the compound with increased biological and pharmacological challenge.

Alternate approaches to in vivo drug evaluation have been prompted by investigations on the metastatic heterogeneity of tumor cell populations. During the 1980s, several investigators associated with NCI conducted studies to assess the metastatic potential of selected murine and human tumor cell lines (B16, A-375, LOX-IMVI melanomas, and PC-3 prostate adenocarcinoma) and their suitability for experimental drug evaluation (e.g., 18–21). A series of investigations by Fidler and associates demonstrated that metastasis is not random, but selective and that metastasis consists of a progression of sequential steps, the pattern of which is dependent on injection site (22,23). Such findings support the importance of establishing in vivo models derived from the implantation of tumor material in host tissues that are anatomically correct—"seed" and "soil" compatibility. Such "orthotopic" models also have been developed and utilized to study lung cancer (e.g., 24), breast cancer (e.g., 25), and prostate cancer (26). Although it may not be possible for NCI to employ these models in the initial steps of in vivo drug evaluations, such models may be well suited for subsequent, more detailed evaluation of compounds that exhibit activity against specific tumor types. Metastases and orthotopic models are discussed in greater detail in Chapters 7 and 8 of this volume.

2.3. Human Tumor Colony Formation Assay, 1981–1985

Based on initial reports by Salmon and colleagues (27,28), various clinical investigators working with fresh human tumor samples from patients and/or with early passage human tumor xenograft materials utilized various culture techniques to identify chemotherapeutic agents active against human malignancies (e.g., 29,30). The NCI sponsored a pilot drug screening project utilizing a human tumor colony-forming assay (HTCFA) at multiple clinical cancer centers. Although it was possible to identify unique antitumor drug "leads" using such a technique, the HTCFA could be employed only for a limited number of tumor types and was not found suitable for large-scale drug screening (31).

2.4. Human Tumor Cell Line Screen, 1985–Present

In 1985, the NCI initiated a new project to assess the feasibility of employing human tumor cell lines for large-scale drug screening (12; also see Chapter 2 of this volume). Cell lines derived from seven cancer types (brain, colon, leukemia, lung, melanoma, ovarian, and renal) were acquired from a wide range of sources, cryopreserved, and subjected to a battery of in vitro and in vivo characterizations, including

testing in drug sensitivity assays. The approach was deemed suitable for large-scale drug screening in 1990 (1). With the implementation of a 60-member cell line in vitro screen, in vivo testing procedures were substantially altered as discussed below.

3. HUMAN TUMOR XENOGRAFT MODELS IN CURRENT USE

The new in vitro human tumor cell line screen shifted the NCI screening strategy from "compound-oriented" to "disease-oriented" drug discovery (12). Compounds of interest identified by the screen (e.g., those demonstrating disease-specific differential cytotoxicity) were to be considered "leads," requiring further preclinical evaluation to determine their therapeutic potential. As part of this followup testing, the antitumor efficacy of the compounds was to be evaluated in in vivo tumor models derived from the in vitro tumor lines used in the screen. Although only a subset of cell lines, selected on the basis of in vitro sensitivity, would be used for each agent, it was anticipated that for any selected compound, any cell line might be required as a xenograft model. In order to accomplish such an objective, a concerted developmental effort was required to establish a battery of human tumor xenograft models. As discussed below and elsewhere (32), tumorigenicity was demonstrated for the majority of the tumor lines utilized in the in vitro screen that became fully operational in April 1990 (1). Then, in 1993, composition of the cell line screen was modified: cell lines with variable growth characteristics and those providing redundant information were replaced by groups of prostate and breast tumor lines. As a consequence, additional xenograft model development was initiated for prostate and breast cancers.

3.1. Development of Human Tumor Xenografts

Efforts focused on the establishment of sc xenografts from human tumor cell-culture lines obtained from the NCI tumor repository at Frederick, MD. The approach is outlined in Fig. 1. The cryopreserved cell lines were thawed, cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (HyClone), and expanded until the population was sufficient to yield $\geq 10^6$ cells. Cells were harvested and then implanted sc into the axillary region of 10 athymic NCr nu/nu mice (1.0×10^7 cells/0.5 mL/mouse) obtained from the NCI animal program, Frederick, MD. Mice were housed in sterile, polycarbonate, filter-capped Microisolator™ cages (Lab. Products, Inc.), maintained in a barrier facility on 12-h light/dark cycles, and provided with sterilized food and water ad libitum. The implanted animals were observed twice weekly for tumor appearance. Growth of the solid tumors was monitored using *in situ* caliper measurements to determine tumor mass. Weights (mg) were calculated from measurements (mm) of two perpendicular dimensions (length and width) using the formula for a prolate ellipsoid and assuming a specific gravity of 1.0 g/cm^3 (33). Fragments of these tumors were subjected to histological, cytochemical, and ultrastructural examination to monitor the characteristics of the in vivo material and to compare them with those of the in vitro lines and, where possible, with those reported for initial patient tumors (34). Both in vitro and in vivo tumor materials exhibited characteristics consistent with tissue type and tumor of origin. However, not unexpectedly, differences in the degree of differentiation were noted between some of the cultured cell lines and corresponding xenograft materials.

The initial solid tumors established in mice were maintained by serial passage of 30–40 mg tumor fragments implanted sc near the axilla. There was an apparent cell

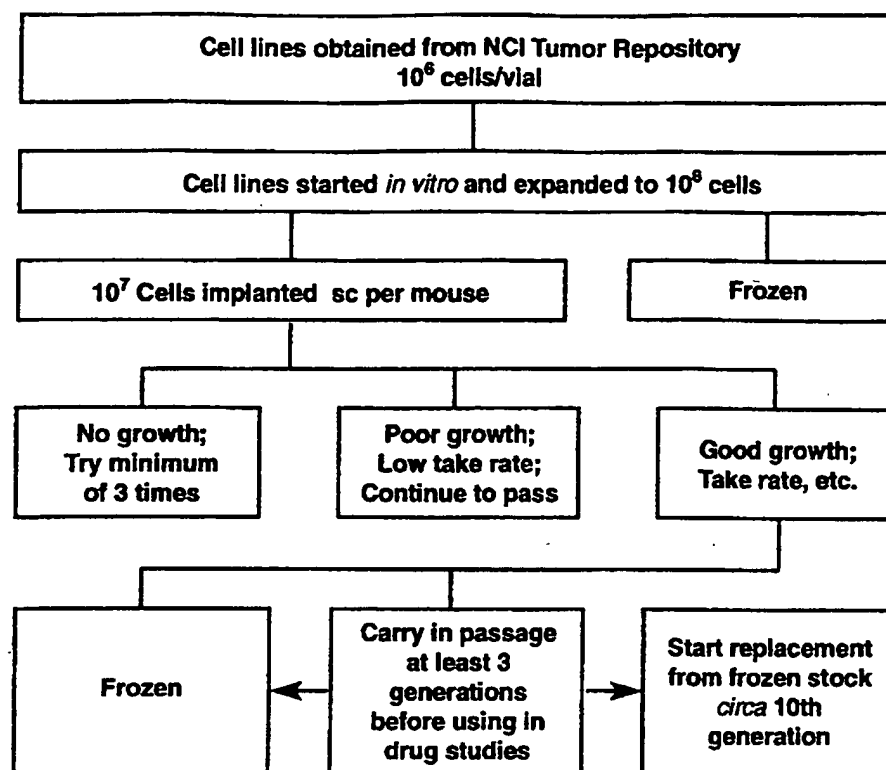


Fig. 1. Schematic of the development of in vivo models for drug evaluation.

population selection occurring in some of the tumors as they adapted to growth in animals during early in vivo passage, with growth rates increasing appreciably in sequential passages (32). Thus, xenografts were not utilized for drug evaluation until the volume-doubling time stabilized, usually around the fourth or fifth passage. The doubling time of xenografts derived from tumor cell lines constituting both the initial (1990) and the modified (1993) human tumor cell line screens, plus three additional breast tumors, are presented in Table 1. Also provided in the table is information on the take-rate of the tumors, and the experience of the NCI in the use of the tumors as early stage sc models. The doubling times were determined from vehicle-treated control mice used in drug evaluation experiments (only data for passage numbers 4–20 have been included). For each experiment, the doubling time is the median of the time interval for individual tumors to increase in size from 200–400 mg (usually a period of exponential growth). Both ranges and mean values are provided to demonstrate the inherent variability of growth for some of the xenograft materials even after a period of stabilization. Mean doubling times range from < 2 d for five tumors (SF-295 glioblastoma, MOLT 4 leukemia, DMS 273 small-cell lung tumor, and LOX-IMVI and SK-MEL-28 melanomas) to > 10 d for the MALME-3M and M19-MEL melanomas.

Difficulty was experienced in establishing and/or using some of the sc models. For example, even though HOP-62 nonsmall-cell lung tumors exhibited good growth rates, poor take-rates of 70, 50, 64, and 30% attained in the second through fifth passages, respectively, precluded their use for experimental drug testing. Although serial passage of OVCAR-3 ovarian tumors from sc-implanted fragments was difficult,

Table 1
Growth Characteristics of sc-Implanted Human Tumor Xenografts

Tumor Origin	Line	<i>In vitro</i> panel status		Mean volume doubling time (range) in days ^a	Take Rate ^b	Opinion for use as early-stage sc model
		1990	1993			
Colon	SW-620	Yes	Yes	2.4(1.7-3.9)	Good	Good
	KM12	Yes	Yes	2.4(1.9-3.3)	Good	Good
	HCT-116	Yes	Yes	2.6(1.8-3.4)	Good	Good
	HCT-15	Yes	Yes	3.4(1.8-5.0)	Good	Good
	HCC-2998	Yes	Yes	3.5(2.4-7.7)	Good	Acceptable
	DLD-1	Yes	No	3.8(3.1-5.5)	Good	Acceptable
	KM20L2	Yes	No	3.9(2.5-5.4)	Good	Acceptable
	COLO 205	Yes	Yes	4.3(2.4-8.9)	Good	Acceptable
	HT29	Yes	Yes	5.1(2.4-7.6)	Good	Acceptable
CNS	SF-295	Yes	Yes	1.4(1.0-2.0)	Good	Good
	SNB-75	Yes	Yes	3.1(2.0-4.6)	Good	Good
	U251	Yes	Yes	4.3(2.4-8.9)	Good	Good
	XF 498	Yes	No	4.4(2.6-8.3)	60-70%	Not Acceptable
	SNB-19	Yes	Yes	6.9(3.1-4.4)	60-70%	Not Acceptable
	SF-539	Yes	Yes	8.4(one only)	70%	Not Acceptable
	SF-268	Yes	Yes	NA ^c	Minimal growth	NA
	SNB-78	Yes	No	NA	No Growth	NA
Leukemia	MOLT-4	Yes	Yes	1.2(2.0-5.6)	80-100%	Acceptable
	HL-60(TB) ^d	Yes	Yes	3.3(2.1-4.9,ip)	85-100%(ip)	Good(ip)
	CCRF-CEM	Yes	Yes	4.6(4.3-4.6)	60-80%	Acceptable
	SR	Yes	Yes	5.1(one only)	80%	Not Acceptable
	RPMI-8226	Yes	Yes	NA	Minimal growth	NA
	K-562	Yes	Yes	NA	Minimal growth	NA
Lung: non-small cell	NCI-H460	Yes	Yes	2.1(1.3-3.0)	Good	Good
	NCI-H522	Yes	Yes	2.3(1.0-3.4)	Good	Good
	HOP-62	Yes	Yes	3.6(3.3-3.8)	30-65%	Not Acceptable
	NCI-H23	Yes	Yes	3.7(2.0-6.4)	Good	Good
	NCI-H322M	Yes	Yes	4.0(2.7-5.9)	Good	Acceptable
	EKVX	Yes	Yes	5.5(3.5-7.9)	Good	Acceptable
	HOP-92	Yes	Yes	6.0(5.1-8.4)	Good	Acceptable
	A549/ATCC	Yes	Yes	8.4(5.8-10.9)	70-80%	Not Acceptable
	HOP-18	Yes	No	NA	Minimal growth	NA
	NCI-H266	Yes	Yes	NA	Minimal growth	NA

tumors grew more readily from sc implants of brei derived from ip-passaged material. The HL-60 (TB) promyelocytic leukemia did not grow well sc, but an ascitic ip line with a good take-rate was established successfully (Table 1). Growth characteristics of sc-implanted RXF 393 renal tumors are perhaps better suited for evaluation of a survival end point than for measurements of tumor size. Although demonstrating

Table 1 (Continued)

Tumor Origin	Line	<i>In vitro</i> panel status		Mean volume doubling time (range) in days ^a	Take Rate ^b	Opinion for use as early-stage sc model
		1990	1993			
Lung: small cell	DMS273	Yes	No	1.7(1.6-2.1)	Good	Good
	DMS114	Yes	No	4.8(2.8-7.5)	75-90%	Acceptable
Mammary	ZR-75-1	No	No	1.8(1.5-1.9)	Good	Good
	MX-1	No	No	2.7(2.2-3.0)	Good	Good
	UIISO-BCA-1	No	No	4.1(2.8-4.8)	Good	Acceptable
	MDA MB-231/ATCC	No	Yes	4.4(2.7-7.7)	Good	Acceptable
	MCF7 ^c	No	Yes	4.5(2.2-8.0)	Good	Acceptable
	MCF7/ADR-RES	No	Yes	6.1(4.2-7.9)	Good	Acceptable
	MDA-MB-435	No	Yes	6.6(2.8-13.6)	Good	Acceptable
	MDA-N	No	Yes	7.9(4.5-10.2)	Good	Acceptable
	HS578T	No	Yes	NA	Minimal growth	NA
	BT-549	No	Yes	NA	No growth	NA
	T-47D	No	Yes	NA	No growth	NA
Melanoma	LOX-IMVI	Yes	Yes	1.5(1.1-2.1)	Good	Good
	SK-MEL-28	Yes	Yes	1.9(1.1-2.5)	Good	Good
	UACC-62	Yes	Yes	2.8(1.8-4.2)	70-80%	Not Acceptable
	UACC-257	Yes	Yes	5.4(3.8-7.7)	Good	Acceptable
	SK-MEL-2	Yes	Yes	5.7(4.8-6.6)	80-90%	Not Acceptable
	M14	Yes	Yes	6.7(2.8-12.7)	Good	Acceptable
	SK-MEL-5	Yes	Yes	7.3(5.1-8.2)	Good	Acceptable
	MALME-3M	Yes	Yes	11.2(7.1-16.9)	80-90%	Not Acceptable
Ovarian	M19-MEL	Yes	No	12.3(8.7-16.8)	60-90%	Not Acceptable
	OVCAR-5	Yes	Yes	3.3(2.2-4.3)	Good	Good
	SK-OV-3	Yes	Yes	3.4(2.6-4.9)	Good	Good
	OVCAR-3 ^c	Yes	Yes	5.5(5.0-5.9)	Good	Acceptable
	OVCAR-4	Yes	Yes	6.2(one only)	70-100%	Acceptable
	IGROV1	Yes	Yes	6.4(5.3-8.6)	Good	Acceptable
	OVCAR-8	Yes	Yes	12.2(11.2-13.0)	70%	Not Acceptable

(continued)

good initial growth, the RXF 393 tumors cause death in mice with low tumor burden, probably owing to paraneoplastic mechanisms. Other cell lines failed to become functional *in vivo* tumors, including two CNS, two nonsmall-cell lung, three breast, three renal tumor lines, and two leukemias, although minimal *in vivo* growth was observed with 9 of these 12 cultured lines (Table 1). With more extensive studies, it might be possible to attain improved tumor take-rates and growth by implanting tumors in

Table 1 (Continued)

Tumor Origin	Line	<i>In vitro</i> panel status		Mean volume doubling time (range) in days ^a	Take Rate ^b	Opinion for use as early-stage sc model
		1990	1993			
Prostate	PC-3	No	Yes	2.4(1.5-3.9)	Good	Good
	DU-145	No	Yes	4.4(2.0-7.9)	Good	Acceptable
Renal	CAKI-1	Yes	Yes	2.1(1.3-2.5)	Good	Good
	RXF 631	Yes	No	3.3(1.5-6.8)	Good	Acceptable
	A498	Yes	Yes	3.4(2.2-4.3)	Good	Acceptable
	RXF 393	Yes	Yes	3.4(2.3-5.7)	Good	Good
	SN12C	Yes	Yes	5.6(3.2-11.4)	Good	Acceptable
	786-0	Yes	Yes	6.7(one only)	80%	Not Acceptable
	ACHN	Yes	Yes	NA	Minimal growth	NA
	UO-31	Yes	Yes	NA	Minimal growth	NA
	TK-10	Yes	Yes	NA ^c	No growth	NA

^aTime for tumors to increase in size from 200–400 mg. Data are compiled from experiments using passage numbers 4–20. Tumors are listed in order of increasing mean doubling time/histologic type.

^bGood: reproducible take-rate of $\geq 90\%$.

^cNA: not applicable.

^dBased on ip implant of 1.0×10^7 cells.

^eMCF7 growth in athymic NCr nu/nu mice requires 17 β -estradiol supplementation.

^fLimited sc data obtained from implant of 0.5 mL 25% brei derived from ip-passaged tumor: poor growth is attained with serial passage of fragments from sc tumors.

severe combined immunodeficient (SCID) mice (scid/scid) (35). As discussed below, tumor take for some human lymphoma lines was markedly superior in SCID mice compared to athymic (nu/nu) (36) or triple-deficient BNX (bg/nu/xid) mice (37).

Establishment of breast tumor xenografts *in vivo* raised issues concerning hormonal requirements for growth of these tumors. For example, the importance of hormones in the growth of MCF7 breast carcinoma cells as solid tumors in athymic mice has been described (38). Our experience with this tumor also has shown the importance of 17 β -estradiol supplementation for the growth of the sc-implanted MCF7; 60-d release, 17 β -estradiol pellets (Innovative Research of America) are implanted sc in athymic mice 24 h prior to implanting MCF7 fragments in all NCI studies with this tumor. Growth of the remaining breast tumor xenografts appeared to be independent of estradiol supplements. For example, growth curves of individual early passage ZR-75-1 tumors implanted into athymic mice receiving either estradiol or no estradiol supplements completely overlapped (Fig. 2A), even though in this early passaged material, there was a large variation in the time postimplant for growth of individual tumors to be observed. The independence of ZR-75-1 tumor growth from estradiol supplements is further illustrated in Fig. 2B. Early stage vehicle-treated control tumors in mice receiving no estradiol supplementation demonstrate rapid growth (median doubling time 1.9 d) soon after implantation. The estrogen receptor (ER) status of the *in vivo* passaged ZR-75-1 tumors has not been determined, but the

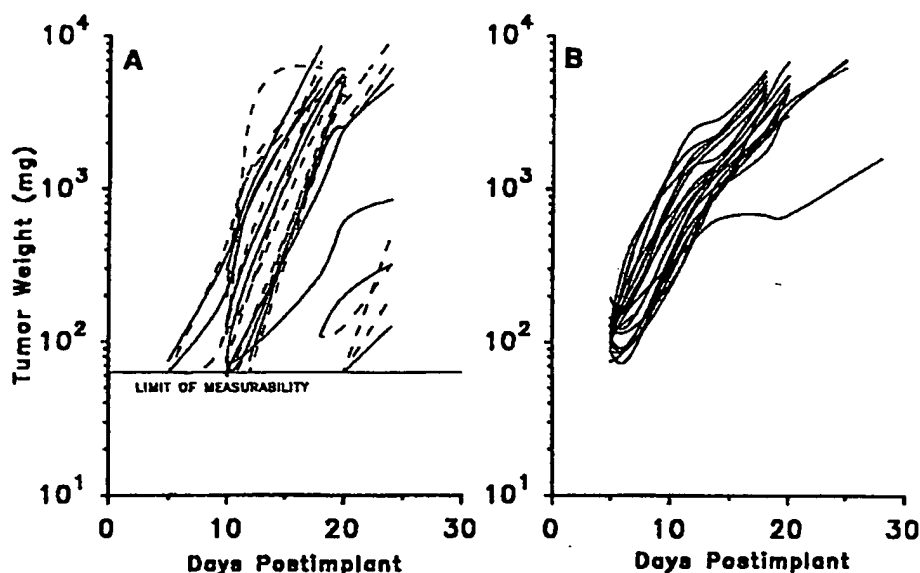


Fig. 2 Growth comparison of individual ZR-75-1 human breast tumor xenografts with and without estradiol supplementation. A. Passage number 5, — with 1.7 mg sc 17 β -estradiol pellet implants; ---without estradiol supplementation. B. Passage number 14, without estradiol supplementation.

growth characteristics of the tumors suggest an ER — status even though early evaluations of the in vitro cell line indicated an ER+ status (39).

The in vivo growth characteristics of the xenografts determine their suitability for use in the evaluation of test agent antitumor activity, particularly when the xenografts are utilized as early stage sc models. For the purposes of the current discussion, the latter model is defined as one in which tumors are staged to 63–200 mg prior to the initiation of treatment. Our experience with the suitability of the xenografts as early stage models is listed in Table 1. Growth characteristics considered in rating tumors include take-rate, time to reach 200 mg, doubling time, and susceptibility to spontaneous regression. As can be noted, the faster-growing tumors tend to receive the higher ratings.

Since non-Hodgkin's lymphoma is one of the two principal malignancies occurring in the growing population of HIV-infected persons (40), the Developmental Therapeutics Program (DTP) has also established a group of human lymphoma xenografts for evaluating potential chemotherapeutic agents (41). This includes AS283, an Epstein-Barr virus (EBV)-positive, HIV-negative Burkitt's lymphoma derived from an AIDS patient (42); KD488, an EBV-negative, pediatric Burkitt's lymphoma (43–46); and RL, a diffuse, small noncleaved B-cell lymphoma (47). These lines grow sc with take-rates in excess of 90% in SCID mice, whereas much lower take-rates occur in athymic or triple-deficient BNX mice. Our finding of greater take-rates for the human leukemias/lymphomas in SCID mice compared to athymic mice is consistent with the known capacity of SCID mice to support xenografts of normal human hematopoietic cells (48).

3.2. Advanced-Stage sc Xenograft Models

Advanced-stage sc-implanted tumor xenograft models were established originally for use in evaluating the antitumor activity of test agents, so that clinically relevant

parameters of activity could be determined, i.e., partial and complete regressions, durations of remission (49-51). Tumor growth is monitored, and test agent treatment is initiated when tumors reach a weight range of 100-400 mg (staging day, median weights approx 200 mg), although depending on the xenograft, tumors may be staged at larger sizes. Tumor size and body weights are obtained approximately 2 times/wk and entered into DTP's DEC 10,000 Model 720 AXP computer. Through software programs developed by staff of the Information Technology Branch of DTP, in particular by David Segal and Penny Svetlik, data are stored, various parameters of effect are calculated, and data are presented in both graphic and tabular formats. Parameters of toxicity and antitumor activity are defined as follows:

1. Parameters of toxicity: Both drug-related deaths (DRDs) and maximum percent relative mean net body weight losses are determined. A treated animal's death is presumed to be treatment-related if the animal dies within 15 d of the last treatment, and either its tumor weight is less than the lethal burden in the control mice, or its net body weight loss at death is 20% greater than the mean net weight change of the controls at death or sacrifice. A DRD also may be designated by the investigator. The mean net body weight of each group of mice on each observation day is compared to the mean net body weight on staging day. Any weight loss that occurs is calculated as a percent of the staging day weight. These calculations also are made for the control mice, since tumor growth of some xenografts has an adverse effect on the weight of the mice.
2. Optimal % T/C: Changes in tumor weight (Δ weights) for each treated (T) and control (C) group are calculated for each day tumors are measured by subtracting the median tumor weight on the day of first treatment (staging day) from the median tumor weight on the specified observation day. These values are used to calculate a percent T/C as follows:

$$\begin{aligned} \% T/C &= (\Delta T / \Delta C) \times 100 \text{ where } \Delta T > 0 \text{ or} \\ &= (\Delta T / T_1) \times 100 \text{ where } \Delta T < 0 \end{aligned} \quad (1)$$

and T_1 is the median tumor weight at the start of treatment. The optimum (minimum) value obtained after the end of the first course of treatment is used to quantitate anti-tumor activity.

3. Tumor growth delay: This is expressed as a percentage by which the treated group weight is delayed in attaining a specified number of doublings (from its staging day weight) compared to controls using the formula:

$$[(T - C) / C] \times 100 \quad (2)$$

where T and C are the median times in days for treated and control groups, respectively, to attain the specified size (excluding tumor-free mice and DRDs). The growth delay is expressed as percentage of control to take into account the growth rate of the tumor since a growth delay based on $T - C$ alone varies in significance with differences in tumor growth rates.

4. Net log cell kill: An estimate of the number of \log_{10} units of cells killed at the end of treatment is calculated as:

$$\{[(T - C) - \text{duration of treatment}] \times 0.301 / \text{median doubling time}\} \quad (3)$$

where the doubling time is the time required for tumors to increase in size from 200-400 mg, 0.301 is the \log_{10} of 2, and T and C are the median times in days for treated and control tumors to achieve the specified number of doublings. If the duration of treat-

ment is 0, then it can be seen from the formulae for net log cell kill and percent growth delay that log cell kill is proportional to percent growth delay. A log cell kill of 0 indicates that the cell population at the end of treatment is the same as it was at the start of treatment. A log cell kill + 6 indicates a 99.9999% reduction in the cell population.

5. Tumor regression: The importance of tumor regression in animal models as an end point of clinical relevance has been propounded by several investigators (49-51). Regressions are defined as partial if the tumor weight decreases to 50% or less of the tumor weight at the start of treatment without dropping below 63 mg (5 X 5 mm tumor). Both complete regressions (CRs) and tumor-free survivors are defined by instances in which the tumor burden falls below measurable limits (< 63 mg) during the experimental period. The two parameters differ by the observation of either tumor regrowth (CR) or no regrowth (tumor-free) prior to the final observation day. Although one can measure smaller tumors, the accuracy of measuring a sc tumor smaller than 4 X 4 or 5 X 5 mm (32 and 63 mg, respectively) is questionable. Also, once a relatively large tumor has regressed to 63 mg, the composition of the remaining mass may be only fibrous material/scar tissue. Measurement of tumor regrowth following cessation of treatment provides a more reliable indication of whether or not tumor cells survived treatment.

Most xenografts that grow sc are amenable to use as an advanced-stage model, although for some tumors, the duration of the study may be limited by tumor necrosis. As mentioned previously, this model enables the investigator to measure clinically relevant parameters of antitumor activity and provides a wealth of data on the effects of the test agent on tumor growth. Also, by staging day, the investigator is ensured that angiogenesis has occurred in the area of the tumor, and staging enables no-takes to be eliminated from the experiment. However, the model can be costly in terms of time and mice. For the slower-growing tumors, the passage time required before sufficient mice can be implanted with tumors may be at least 3-4 wk, and an additional 2-3 wk may be required before the tumors can be staged. In order to stage tumors, more mice than needed for actual drug testing must be implanted, often 50%, and sometimes 100% more.

3.3. Early Treatment and Early Stage sc Xenograft Models

Early treatment and early stage sc models are similar to the advanced-stage model, but because treatment is initiated earlier in the development of the tumor, the models are not suitable for tumors that have less than a 90% take-rate or have a > 10% spontaneous regression rate. We define the early treatment model as one in which treatment is initiated before tumors are measurable, i.e., < 63 mg, and the early stage model as one in which treatment is initiated when tumor size ranges from 63-200 mg. The 63-mg size is used as an indication that the original implant of approx 30 mg has demonstrated some growth. Parameters of toxicity are the same as those for the advanced-stage model; parameters of antitumor activity are similar. Percent T/C values are calculated directly from the median tumor weights on each observation day instead of as changes (Δ) in tumor weights, and growth delays are based on the time in days after implant for the tumors to reach a specified size, e.g., 500 or 1000 mg. Tumor-free mice are recorded, but may be designated no-takes or spontaneous regressions if the vehicle-treated control group contains more than 10% mice with similar growth characteristics. A no-take is a tumor that fails to become established and grow progressively. A spontaneous regression (graft failure) is a tumor that, after

a period of growth, decreases to 50% or less of its maximum size. Tumor regressions are not normally recorded, since they are not always a good indicator of antineoplastic effects in the early stage model. For those experiments in which treatment is initiated when tumors are 100 mg or less, only a minimal reduction in tumor size may bring the tumor below the measurable limit, and for some small tumors early in their growth, reductions in tumor size may reflect erratic growth rather than a true reflection of a cell killing effect. The big advantage of the early treatment model is the ability to use all implanted mice. The latter is the reason for requiring a good tumor take-rate, and in practice, the tumors most suitable for this model tend to be the faster-growing ones.

3.4. Challenge Survival Models

Although not utilized to a significant degree in the current NCI program, a few studies are conducted that depend on determining the effect of human tumor growth on the life-span of the host. Three tumors have been used as ip-implanted models: the HL-60 (TB) promyelocytic leukemia, the LOX-IMVI melanoma, and the OVCAR-3 ovarian carcinoma. Also, the SF-295 and U251 glioblastomas have been implanted intracerebrally. All mice dying or sacrificed owing to a moribund state or extensive ascites prior to the final observation day are used to calculate median days of death for treated (T) and control (C) groups. These values are then used to calculate a percent increase in life-span as follows:

$$\% \text{ ILS} = [(T - C)/C] \times 100 \quad (4)$$

Wherever possible, titration groups are included to establish a tumor doubling time for use in \log_{10} cell kill calculations. Laboratory personnel may designate a death (or sacrifice) as drug-related based on visual observations and/or the results of necropsy. Otherwise, treated animal deaths are designated as treatment-related if the day of death precedes the mean day of death of the controls minus 2 SD or if the animal dies without evidence of tumor within 15 d of the last treatment.

3.5. Response of Xenograft Models to Standard Agents

The drug sensitivity profiles for the advanced-stage sc xenograft models in our program have been established using 12 clinical antitumor drugs (Table 2). Each of these agents, obtained from the Drug Synthesis and Chemistry Branch, DTP, were evaluated following ip administration at multiple dose levels. The activity ratings are based on the optimal effects attained with the maximally tolerated dose ($<LD_{20}$) of each drug for the treatment schedule shown. The latter were selected on the basis of the doubling time of a given tumor, with longer intervals between treatments for slower-growing tumors. Apparent inconsistencies between the doubling times shown in Table 1 and selected schedules in Table 2 are the result of increased tumor growth rates for some tumors in the later studies depicted in Table 1. In later chemotherapeutic trials with breast tumors, paclitaxel was included in the group of clinical drugs evaluated, and drug characteristics were considered to some extent in the selection of treatment regimens (Table 3).

With the caveat that no attempts were made to optimize drug administration in each model, it can be seen that at least minimal antitumor effects ($\%T/C \leq 40$) were

Table 2
Response of Staged sc-Implanted Human Tumor
Xenografts to 12 Clinical Anticancer Drugs^b

Tumor	IP Treatment Schedule	Alkylating Agents						DNA Binders			Antimetabolites		Mitotic Inhibitor
		L-PAM	CYT	DTIC	BCNU	MMC	DDP	Act D	ADR	BLFO	MTX	SFU	VEL
Colon:													
SW-620	q7dx3	1	0	4	3	4	1	0	0	1	0	0	0
KM12	q4dx3	NA	NA	0	1	NA	0	0	NA	1	0	0	0
HCT-116	q4dx3	NA	0	0	0	1	0	1	NA	1	0	0	0
HCT-15	q7dx3	1	0	0	0	1	0	1	0	0	0	0	0
HCC-2998	q4dx3	0	0	0	0	4	0	0	0	1	0	0	0
KM20L2	q4dx3	0	1	0	0	1	1	0	0	0	0	1	0
COLO 320DM	q4dx3	0	0	3	0	0	NA	0	0	0	0	1	0
COLO 205	q4dx3	1	0	0	0	3	1	1	0	0	0	1	1
HT29	q4dx3	0	0	0	0	2	2	0	0	1	0	0	0
CNS:													
SF-295	q4dx3	0	1	0	1	0	1	0	0	0	0	0	4
U251	q4dx3	0	2	4	4	3	3	1	0	0	0	0	1
XF 498	q7dx3	0	0	4	3	1	1	0	1	0	0	0	0
SNB-19	q4dx3	1	2	3	NA	3	NA	0	NA	NA	0	0	1
Leukemia:													
MOLT-4	q4dx3	4	1	0	0	1	1	0	1	1	1	0	0
Lung, non-small cell:													
NCI-H460	q4dx3	NA	0	NA	NA	4	NA	0	0	1	NA	0	0
NCI-H522	q4dx3	1	0	0	NA	≥2	1	NA	1	NA	NA	0	NA
HOP-62	q4dx3	2	NA	NA	NA	1	1	0	0	1	0	0	1
Lung, non-small cell:													
NCI-H23	q4dx3	3	1	0	0	4	4	1	1	1	1	1	0
NCI-H322M	q7dx3	0	0	0	0	4	1	0	0	0	0	1	0
EKVX	q4dx3	1	1	1	0	1	0	0	0	1	0	0	1
HOP-92	q4dx3	1	0	4	2	0	1	0	1	0	0	0	1
Lung, small cell:													
DMS 273	qdx4	1	1	0	0	1	0	1	0	1	1	1	2
DMS 114	q4dx3	0	1	0	1	1	0	0	0	0	0	0	1
NCI-H69	q4dx3	4	1	3	4	2	NA	0	NA	0	0	1	0
Melanoma:													
LOX-IMVI	qdx5	1	2	2	2	2	1	0	NA	0	NA	0	2
SK-MEL-28	q4dx3	0	1	0	0	1	0	0	0	0	0	0	0
UACC-62	q7dx3	0	0	1	1	1	1	1	1	1	0	0	1
SK-MEL-31	q4dx3	0	0	0	NA	1	1	1	0	NA	0	0	NA
UACC-257	q7dx3	0	0	4	1	2	1	1	1	0	0	1	0
SK-MEL-2	q7dx3	1	0	0	0	1	1	1	0	1	0	1	2
M14	q4dx3	0	0	0	0	0	0	0	1	1	0	1	0
MALME-3M	q4dx3	1	0	4	1	1	1	1	0	1	0	0	0
Ovarian:													
SK-OV-3	q7dx3	0	0	NA	NA	1	0	0	0	1	0	0	0
IGROV1	q4dx3	1	0	0	0	1	1	1	0	1	1	1	0
OVCAR-8	q7dx3	0	0	0	0	1	1	0	1	0	0	0	0

(continued)

produced in each tumor model by at least 2, and as many as 10, clinical drugs (Table 4). The number of responses appeared to be independent of doubling time and histological type with a range in the number of responses observed for tumors in each sub-panel. When the responses are considered in terms of the more clinically relevant end points of partial or complete tumor regression, it can be seen that the tumor models were quite refractory to standard drug therapy with 30 of 48 (62.5%) not responding

Table 2 (Continued)

Tumor	IP Treatment Schedule	Alkylating Agents						DNA Binders			Antimetabolites		Mitotic Inhibitor VBL
		L-PAM	CYT	DTIC	BCNU	MMC	DDPt	Act D	ADR	BLEO	MTX	5FU	
Prostate:													
PC-3	qdx4	1	0	1	0	0	0	0	0	1	1	0	0
DU-145	q4dx3	NA	1	NA	NA	NA	1	0	NA	NA	1	0	0
Renal:													
CAKI-1	q7dx3	1	1	0	0	1	1	1	1	0	0	1	0
SN12K1	q7dx3	0	0	0	0	4	0	0	0	0	0	0	1
A498	q7dx3	0	0	1	1	1	1	0	1	1	0	0	0
RXF 393	q4dx3	1	1	1	1	2	1	0	1	1	0	0	1
SN12C	q7dx3	0	0	0	1	0	0	0	0	1	0	0	0
786-0	q7dx3	0	NA	NA	NA	1	1	0	NA	NA	0	NA	NA

^aStandard agents are melphalan (L-PAM), cytoxan (CYT), dacarbazine (DTIC), 1, 3-bis (2-chloroethyl)-1-nitrosourea (BCNU), mitomycin C (MMC), cisplatin (DDPt), actinomycin D (act D), doxorubicin (ADR), bleomycin (BLEO), methotrexate (MTX), 5-fluorouracil (5FU), vinblastine (VBL).

^bActivity rating based on optimal %Δ T/Δ C attained after treatment had ended:

0 = Inactive, %T/C > 40.

1 = Tumor inhibition, %T/C range 1-40.

2 = Tumor stasis, %T/C range 0 to -49.

3 = Tumor regression, %T/C range -50 to -100.

4 = %T/C range -50 to -100 and >30% tumor-free mice at experiment end.

Table 3
Response of Staged sc-Implanted Breast Tumor
Xenografts to 13 Clinical Anticancer Drugs^a

Tumor ^b		Alkylating Agents						DNA Binders			Antimetabolites		Mitotic Inhibitor	
		L-PAM	CYT	DTIC	BCNU	MMC	DDPt	Act D	ADR	BLEO	MTX	5FU	VBL	PAC
ZR-75-1	1 ^b	1	4	1	1	1		1	3	1	0	0	1	NA
MX-1	4	4	0	1	4	4		0	2	1	1	0	0	4
UISO-BCA-1	0	0	1	0	NA	0		0	0	0	NA	0	0	3
MCF7	1	0	1	1	1	0		0	0	1	0	NA	0	0
MDA-MB-435	1	0	4	3	NA	0		NA	0	1	NA	0	1	4
MDA-N	0	0	4	0	0	0		1	1	0	0	NA	0	3

^aClinical drugs include those listed in Table 2, plus paclitaxel (PAC). Treatment regimens were ip qd x 5 for MTX and 5FU, ip q4d x 3 for L-PAM, CYT, DTIC, BCNU, MMC, DDPt, Act D, BLEO, and VBL, iv q4d x 3 for ADR, and iv qd x 5 for PAC.

^bActivity rating; see legend to Table 2.

to any of the drugs tested (Table 4). As tested, the clinical drugs producing the highest response rates (number of tumors responding [%T/C ≤ -50%]/total tumors evaluated) were DTIC (11/44) and mitomycin C (9/45) (Tables 2 and 3). Paclitaxel was not evaluated in the majority of tumors, but demonstrated excellent activity in four of five breast tumor models (Table 3).

Table 4
Response of Staged sc Human Tumor Xenografts to Clinical Anticancer Drugs

Panel	Tumor	Number of drugs active ^a :	
		Minimal activity ^b	Tumor regression ^c
Colon	SW-620	6	3
	KM12	2/8	0/8
	HCT-116	3/10	0/10
	HCT-15	3	0
	HCC-2998	2	1
	KM20L2	4	0
	COLO 320DM	2/11	0/11
	COLO 205	6	1
	HT29	3	0
CNS	SF-295	4	1
	U251	7	4
	XF 498	5	2
	SNB-19	5/8	2/8
Lung, non-small cell	NCI-H460	2/7	1/7
	NCI-H522	4/7	0/7
	HOP-62	5/9	0/9
	NCI-H23	9	3
	NCI-H322M	3	1
	EKVX	6	0
	HOP-92	6	1
Lung, small cell	DMS 273	8	0
	DMS 114	4	0
	NCI-H69	6/10	3/10
Mammary	ZR-75-1	10/12	2/12
	MX-1	9	5
	UIISO-BCA-1	2/11	1/11
	MCF7	5/12	0/12
	MDA-MB-435	6/10	3/10
	MDA-N	4/12	2/12

(continued)

3.6. Strategy for Initial Compound Evaluation In Vivo

The in vitro primary screen provides the basis for selection of the most appropriate lines to use for the initial followup in vivo testing, with each compound tested only against xenografts derived from cell lines demonstrating the greatest sensitivity to the

Table 4 (Continued)

Panel	Tumor	Number of drugs active ^a	
		Minimal activity ^b	Tumor regression ^c
Melanoma	LOX-IMVI	7/10	0
	SK-MEL-28	2	0
	UACC-62	8	0
	SK-MEL-31	3/9	0/9
	UACC-257	7	0
	SK-MEL-2	7	0
	M14	3	0
	MALME-3M	7	1
Ovarian	SK-OV-3	2/10	0/10
	IGROV1	7	0
	OVCAR-8	3	0
Prostate	PC-3	4	0
	DU-145	3/6	0/6
Renal	CAKI-1	7	0
	SN12K1	2	0
	A498	6	0
	RXF 393	9	0
	SN12C	2	0
	786-0	2/5	0/5

^aExcept where noted, the number of clinical drugs evaluated was 12 for the tumors listed in Table 2, and 13 for the breast tumors listed in Table 3.

^b% T/C \leq 40, ratings 1-4 in Tables 2 and 3.

^c% T/C \leq -50, ratings 3 and 4 in Tables 2 and 3.

agent in vitro. Our early strategy for in vivo testing emphasized the treatment of animals bearing advanced-stage tumors. Examples of the in vivo data obtained with one such agent are summarized in Table 5. The quinocarmycin derivative DX-52-1, identified as a melanoma-specific agent in vitro, demonstrated statistically significant antitumor activity against five of seven melanoma xenografts following ip administration on intermittent schedules (52). The best in vivo activity was observed against the rapidly dividing LOX-IMVI melanoma.

The strategy for in vivo testing has undergone some modifications as experience has been gained with the screen and the xenograft models. Currently, dose range finding studies in nontumored mice are conducted for new compounds identified by the in vitro screen. Unless information is available to guide dose selection, single mice are treated with single ip bolus doses of 400, 200, and 100 mg/kg and observed for 14 d. Sequential three-dose studies are conducted as necessary, until a nonlethal dose range is established, after which the compound is evaluated in the ip-implanted murine P388 leukemia model on an ip multidose treatment regimen. The latter provides dos-

Table 5
Response of Advanced-Staged sc Human Melanoma
Xenografts to the Quinocarmycin Derivative, DX-52-1^a

Melanoma	Treatment days	Dose (ip, mg/kg/day) ^b	Optimal %T/C (Day) ^c	Growth delay: %(T-C)/C ^d	<u>Regressions</u> Complete - Partial	
LOX-IMVI	5, 9, 13	90	-54	181	2/10	3/10
	5, 9, 13, 17, 21, 25	60	-100 ^c	389	4/6	0/6
SK-MEL-2	14, 21, 28	90	10(32)	118	2/6	0/6
SK-MEL-5	15, 19, 23	40	49(33)	27	0/6	0/6
UACC-62	16, 23, 30	90	18(34)	185	0/7	0/7
UACC-257	16, 20, 24	90	12(27)	35	0/6	0/6
M14	12, 16, 20	90	19(26)	56	0/6	0/6
MALME-3M	27, 31, 35	90	64(72)	4	0/6	0/6

^aAdapted from ref. 52.

^bMaximally tolerated dose, \leq LD₁₀ and $<$ 20% net body wt loss.

^cSee Section 3.2., Step 2 for calculation of % T/C. The number in parenthesis is the day on which the optimal (minimum) T/C was attained.

^dSee Section 3.2., Step 3 for calculation of growth delay. Based on an end point of two doublings (four for LOX-IMVI).

ing information for the more costly xenograft models and data for retrospective comparison with previous NCI screening. The test agent is then evaluated in three sc xenograft models using tumors that were among the most sensitive to the test agent in vitro and that are suitable for use as early staged models (Table 1). The compounds are administered ip, often as suspensions, on schedules based, with some exceptions, on the mass doubling time of the tumor. For doubling times of 1.3–2.5, 2.6–5.9, and 6–10 d, the schedules are daily for five treatments (qd \times 5), every fourth day for three treatments (q4d \times 3), and every seventh day for three treatments (q7d \times 3). For most tumors, the interval between individual treatments approximates the doubling time of the tumors, and the treatment period allows a 0.5–1.0 log₁₀ unit of control tumor growth. For tumors staged at 100–200 mg, the tumor sizes of the controls at the end of treatment range from 500–2000 mg, which allows sufficient time after treatment to evaluate the effects of the test agent before it becomes necessary to sacrifice mice owing to tumor size.

Table 6
Effect of Route of Administration on the Activity
of Paclitaxel Against Staged sc-Implanted MX-1 Mammary Carcinoma Xenografts

Treatment		Complete Regressions /Total	Tumor- Free on Day 40	Minimum %T/C ^b (Day)	Growth Delay: %(T-C)/C ^c	Net Log Cell Kill
Route, Schedule	Opt. Dose (mg/kg/day) ^a					
iv, D8-12	22.5	1/9	8	-100(15) ^d	449	2.9
ip, D8-12	15.0	2/9	1	28(19)	29	-0.2
iv, D8,12,16	22.5	6/9	2	-100(15)	357	2.1
ip, D8,12,16	30	0/9	0	20(22)	70	-0.3

^aPaclitaxel was administered as a solution in 12.5% ethanol: 12.5% cremophor: 75% normal saline at multiple dose levels. Data from doses (\leq LD₅₀) producing the optimal effects are shown.

^bSee Section 3.2. for an explanation of the parameters of antitumor effects.

^cBased on an end point of two doublings, C = 7.1 d. Tumor-free mice and mice dying of apparent drug-related effects were excluded from the calculations.

^dNumber in parentheses indicates the observation day on which data were obtained.

3.7. Detailed Drug Studies

Once a compound has been identified that demonstrates some in vivo efficacy in initial evaluations, more detailed studies can be designed and conducted in human tumor xenograft models to explore further the compound's therapeutic potential. By varying the concentration and exposure time of the tumor cells and the host to the drug, it is possible to devise and recommend treatment strategies designed to optimize antitumor activity. As many of the initial in vivo studies deliver suspensions of the compound into the peritoneal cavity, it is unlikely that the sc tumors receive optimal concentrations of, and exposure to, test agents. The early preclinical antitumor evaluation of paclitaxel illustrates this problem. In NCI studies, prior to its clinical evaluation, paclitaxel had demonstrated its best effects against ip-implanted tumors, and no activity was observed in sc models following ip administration as a suspension (53, 54). Later investigations demonstrated that sc-implanted MX-1 mammary carcinoma xenografts were highly responsive to treatment with iv solutions of paclitaxel (55), although they had failed to respond to treatment with ip suspensions (54). As illustrated in Table 6, iv solutions of paclitaxel administered on either a daily or intermittent schedule produced complete tumor regressions in the majority of the treated mice. Some of these mice remained tumor-free 24-28 d after the last treatment, and tumor growth delays in the remaining mice were excellent. In contrast, only modest antitumor effects were observed following the ip administration of paclitaxel solutions. Pharmacokinetic data obtained in mice indicated only 10% bioavailability of paclitaxel from ip administration (56).

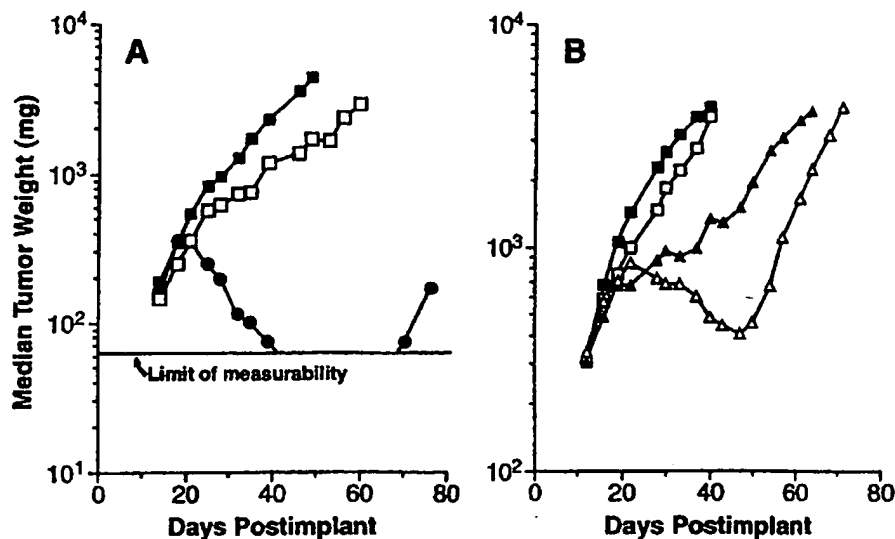


Fig. 3 Response of advanced-stage sc human HT29 colon tumor xenografts to 9-amino-20(S)-camptothecin (9-AC). **A.** ■—■, Vehicle-treated (saline:tween 80) controls; ●—●, 4 mg/kg/d administered as an sc bolus suspension in saline:tween 80; □—□, 4 mg/kg/d administered as an sc bolus solution in propylene glycol (PG):polyethylene glycol 400 (PEG 400): DMSO (95% of an 80% PG: 20% PEG mixture + 5% DMSO). Treatments were administered on a q4d x 8 schedule starting 14 d postimplant. **B.** ■—■, Vehicle-treated (saline:tween 80) controls; □—□, 4 mg/kg/d sc q4d x 8; ▲—▲, 3 mg/kg/d, sc q2d x 16; △—△, 1 mg/kg/d, sc qd x 32. All 9-AC treatments were given as solutions in the PG:PEG:DMSO vehicle starting on day 12.

The importance of “concentration x time” on the antitumor effects of test agents is well illustrated by data obtained with 9-amino-20(S)-camptothecin (9-AC). Although an sc bolus injection of a 9-AC suspension caused complete tumor regression of advanced-stage sc-implanted human HT29 colon tumor xenografts, only a marginal growth-inhibitory effect was observed when 9-AC was administered as a solution (Fig. 3A). Augmented activity with the solution was obtained by increasing the frequency of administration (Fig. 3B). Characterization of the plasma pharmacokinetics in mice, conducted in conjunction with the efficacy studies, indicated that absorption of 9-AC from sc-injected solutions was rapid and efficient, but elimination also was fast (57). The plasma profile afforded from the suspension differed profoundly: peak plasma levels were lower and the rate of elimination much slower (57). The studies indicated that maintaining the 9-AC lactone plasma concentration above a threshold level for a prolonged period of time was required for optimal therapeutic effects.

4. HOLLOW-FIBER ASSAYS: A NEW APPROACH TO IN VIVO DRUG TESTING

DTP is evaluating the suitability of a model that would be integrated into the present drug development scheme between the in vitro 60 cell line screen and the in vivo sc xenograft assays. This model, based on human tumor cell lines growing in hollow fibers, is being developed as a prioritization tool through which lead compounds

identified in the *in vitro* screen would pass (58). The goal is to direct the most promising compounds into *in vivo* testing as rapidly as possible after their selection for testing from the *in vitro* screening data. Presently, 10,000 compounds are screened *in vitro* each year and 8–10% of these are referred for *in vivo* testing. Current resources provide testing for approx 300–350 compounds in the xenograft models annually. Thus, the *in vivo* evaluation of compounds for activity is limited by the availability of testing resources. A method for prioritizing compounds for testing in the xenograft models, and for identifying the most sensitive cell lines to use in the models, would increase the prospect of rapidly identifying those compounds with the greatest potential for having *in vivo* efficacy. The hollow-fiber assay is intended to serve this purpose. In brief, tumor cells are inoculated into hollow fibers (1-mm internal diameter), and the fibers are heat-sealed and cut at 2-cm intervals. These samples are cultivated for 24–48 h *in vitro* and then implanted into athymic (nu/nu) mice. At the time of implantation, a representative set of fibers is assayed for viable cell mass by the “stable-end point” MTT dye conversion technique (59) in order to determine the time zero cell mass for each cell line. The mice are treated with experimental therapeutics on a daily treatment schedule, and the fibers are collected 6–8 d postimplantation. At collection, the quantity of viable cells contained in the fibers is measured. The antitumor effects of the test agent are determined from the changes in viable cell mass in the fibers collected from compound-treated and diluent-treated mice. Using this technique, three different tumor cell lines can be grown conveniently in each of two physiologic sites (ip and sc) within each experimental mouse (58). Thus, this model provides a method whereby a test agent can be administered ip to evaluate its effect against tumor cells growing in both the ip cavity and the sc compartment. With this simultaneous assessment of multiple tumor cell lines grown in two physiologic compartments, it is possible to identify lead compounds rapidly with the greatest promise of *in vivo* activity.

A graphic representation of typical data generated with this assay is shown in Fig. 4. The experimental mice received an ip and an sc hollow fiber of each of three human cell lines (COLO 205 colon, U251 CNS, and OVCAR-5 ovarian tumors) being evaluated. The mice were treated ip with the test agent on days 1–4, and the hollow fibers were removed on day 6. The viable cell mass was determined for the fiber samples, and the percent net growth of each cell line was calculated with reference to the time zero viable cell mass present in the hollow fibers on the day of implantation into mice. The net growth of each cell line in the ip and sc fibers is shown in Fig. 4A and B, respectively. The test agent was effective in suppressing growth of all three cell lines with the greater activity measured against ip implants. Of the cell lines tested, COLO 205 was the most sensitive, a finding that was confirmed by the results of the sc xenograft evaluations conducted following the hollow-fiber assay.

Experimentation to date indicates that this *in vivo/in vitro* hollow-fiber system may be well suited for the prioritization of compounds for more advanced stages of *in vivo* drug evaluation. In a practical sense, this hollow-fiber system is viewed as a means to facilitate traditional chemotherapeutic testing, since it is rapid, appears to be sensitive, and is broadly applicable to a variety of human tumor cell types. Additionally, it requires only a limited quantity of test compound, a small number of animals, and very limited animal housing space.

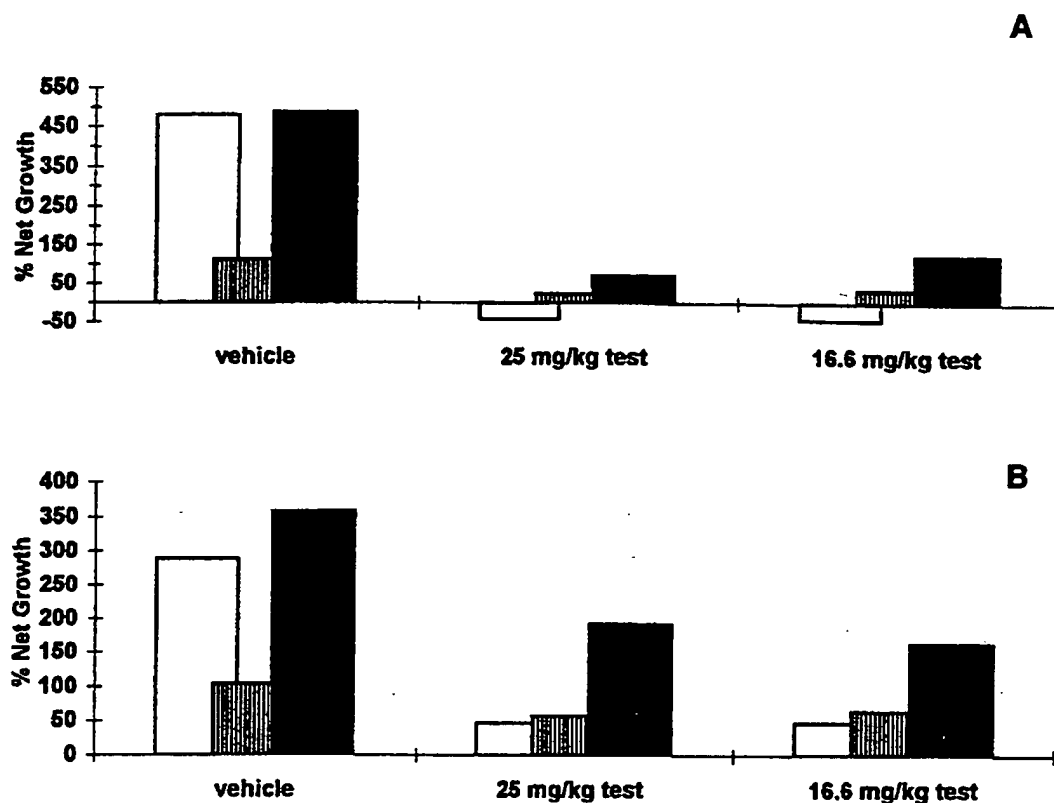


Fig. 4 In vivo effects of test agents on growth of human tumor cells in hollow fibers. Cell lines are COLO 205 colon, open bars; U251 glioblastoma, striped bars; and OVCAR-5 ovarian; solid bars. A. ip Implants. B. sc Implants. Test agents was administered ip on days 1–4, and fiber contents were assayed on day 6.

5. SUMMARY

The discovery and development of potential anticancer drugs by NCI are based on a series of sequential screening and detailed testing steps to identify new, efficacious lead compounds and to eliminate inactive and/or highly toxic materials from further consideration. Past experience in large-scale screening with a wide variety of animal and human tumor systems and the management of disease-free athymic mouse facilities has proven to be highly valuable for the recent characterization, calibration, and utilization of newly acquired human tumor xenograft models. Furthermore, DTP's experience with computer programming has enabled the development and implementation of specialized analytical software, which permits acquisition, storage, and presentation of data in readily accessible tabular and graphic formats. Many of the human tumor xenografts have been employed to test a variety of distinct chemical compound classes over the past five years. Thus, the in vivo drug sensitivity profiles of these human tumor xenografts are well suited to serve as "benchmarks" for the testing of newly synthesized agents as well as agents isolated from natural product sources that are currently under investigation.

In addition to standard models of in vivo testing, NCI has also utilized human tumor materials to explore the suitability of alternate model systems, e.g., "metastasis" and "orthotopic" models. At present, our program is investigating the utility of a capillary hollow-fiber implantation model as a means to prioritize compounds active in the in vitro screen for subsequent in vivo evaluations. It is hoped that further refinement and application of the procedures described in this chapter will facilitate the identification and preclinical development of more efficacious treatments for human malignancy.

ACKNOWLEDGMENTS

The authors thank Jacquelyn Tubbs, Bonnie Beall, Katherine Gill, and Rebecca Brown for their invaluable assistance in assembling data and typing.

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